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Organic contaminants in distilled sugar cane spirits produced by column and copper alembic distillation

Quantificação de contaminantes orgânicos em aguardentes de cana/cachaça produzidas por destilação em coluna e de alambique de cobre

Cuantificación de contaminantes orgánicos en aguardientes de cana/cachaza producidos por destilación en columna y todavía cobre

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Abstract

The present work aimed to characterize and quantify four contaminants (ethyl carbamate, 2,3-butanedione, furfural and 5-hydroxymethylfurfural) present in still and industrial cachaça. The four contaminants ethyl carbamate, 2,3-butanedione, furfural (FU) and 5-hydroxymethylfurfural (5-HMF) were analyzed in spirits produced by alembic and column distillation. Forty-four samples of cachaça were collected in the southern, central-western, and southeastern regions of the state of Minas Gerais and in the state of São Paulo. The samples were subjected to chromatographic analysis. Ethyl carbamate, 2,3-butanedione, furfural and 5-HMF were characterized and quantified by HPLC. Two samples of spirits were found to contain concentrations of ethyl carbamate that were greater than the legal limits, ranging from 245.31 to 235.53 μg L^{-1}. None of the alembic samples had concentrations higher than the legal limit. The spirits obtained by column disitllation contained higher concentrations of the 2,3-butanedione than the alembic liquors. An analytical method was developed and validated for the quantification of furfural and 5-HMF, and the spirits obtained by column distillation contained concentrations higher than the limit established by legislation.

Keywords: Sugar cane; Drinks; Quality; Contaminants.

Resumo

No presente trabalho objetivou-se caracterizar e quantificar quatro contaminantes (carbamato de etila, 2,3-butanodiona, furfural e 5-hidroximetilfurfural) presentes em cachaça de alambique e industrial. Os quatro contaminantes carbamato de etila, 2,3-butanodiona, furfural (FU) e 5- hidroximetilfurfural (5-HMF) foram analisados em bebidas produzidas por destilação de alambique e coluna. Quarenta e quatro amostras de cachaças foram coletadas nas regiões sul, centro-oeste e sudeste dos Estados de Minas Gerais e São Paulo. O carbamato de etila, 2,3-butanodiona, furfural e 5-HMF foram caracterizados e quantificados por HPLC. Verificou-se que duas amostras de bebidas continham concentrações de carbamato de etila superiores aos limites legais, variando de 245,31 a 235,53 μg L-1. Nenhuma das amostras de alambique apresentou concentrações superiores ao limite legal. As bebidas obtidas pela desililação da coluna continham concentrações mais altas de 2,3-butanodiona do que as cachças de alambique. Um método analítico foi desenvolvido e validado para a quantificação

de furfural e 5-HMF, e bebidas obtidas por destilação em coluna continham concentrações superiores ao limite estabelecido pela legislação.

Palavras-chaves: Cana de açucar; Bebidas; Qualidade; Contaminantes.

Resumen

El presente trabajo tuvo como objetivo caracterizar y cuantificar cuatro contaminantes (carbamato de etilo, 2,3-butanodiona, furfural y 5-hidroximetilfurfural) presentes en la cachaça industrial e inmóvil. Fueron analizados cuatro contaminantes en bebidas producidas por destilación de alambique y columna: carbamato de etilo, 2,3-butanodiona, furfural (FU) y 5-hidroximetilfurfural (5-HMF). Cuarenta y cuatro muestras de aguardientes (conocidas popularmente en el Brasil como “cachaça”, cachaza) se recolectaron en las regiones sur, medio oeste y sudeste de los estados de Minas Gerais y São Paulo (Brasil). Las muestras fueron sometidas a análisis cromatográfico y el carbamato de etilo, la 2,3-butanodiona, el furfural y el 5-HMF se caracterizaron y cuantificaron por la técnica de cromatografía líquida de alto desempeño (HPLC). Se encontró que dos muestras de bebidas contenían concentraciones de carbamato de etilo entre 245,31 y 235,53 μg L-1, valores por encima de los límites legales. Ninguna de las muestras de alambique mostró concentraciones superiores al límite legal. Las bebidas obtenidas de la destilación de columna contenían concentraciones de 2,3-butanodiona más altas que aquellas obtenidas por la técnica de alambique. Se desarrolló y validó un método analítico para la cuantificación de furfural y 5-HMF, y las bebidas obtenidas por destilación de columna contenían concentraciones superiores al límite establecido por la legislación vigente.

Palabras clave: Caña de azúcar; Calidad; Bebidas; Contaminantes.

1.Introduction

Cane spirits are one of the most widely consumed distillates in Brazil. They are obtained from fermented cane juice, and the name varies according to the region of the country. The annual production is approximately 1.6 billion liters, of which 90% is produced industrially, and 10% from alembic stills. The state of Minas Gerais stands out as the largest producer of alembic spirits in the country, representing 44% of the national production. Currently, projects are being developed that seek to improve the quality of the product by improving manufacturing practices that result in standardized products with a proven physicochemical and sensorial quality. However, the production of high quality cane spirits requires technical and scientific improvement of the production stages with research in

genetic improvement of sugarcane, harvesting, management, fermentation, distillation and aging (Sebrae, 2014).

The requirements of the internal and external markets signify that improvements in its quality should be implemented, not only from the commercial point of view, but mainly considering the toxicological effects, because a product that contains undesirable compounds can be detrimental to the health of the consumer. In the present study, samples of sugar cane spirits produced via column distillation and via simple distillation from copper alembics in the southern, central-western, and southeastern regions of the state of Minas Gerias and in the state of São Paulo were analyzed for the presence of contaminants such as ethyl carbamate, furfural (FU), 5-hydroxymethylfurfural (5-HMF) and 2,3-butanedione. The analytical method for the quantification of furfural and 5-HMF, which are compounds that might be formed or transferred at some stage in the production process, was also validated.

2. Material and Methods

2.1 Sample Collection

The spirits used were collected randomly in various regions of the State of Minas Gerais (South, Midwest and Southeast) and in the State of São Paulo in the period from 2014 to 2015. 44 samples were collected in different commercial production units. The samples were coded according to the distillation process: 15 samples were distilled through a stainless steel column (A1 - A15) and 29 samples were distilled in copper stills (A16 - A44). Samples A3, A12, A20, A21, A32, A33, A38, A39 and A44 were aged in cachaça. Chromatographic analyzes were carried out at the Brandy Quality Analysis Laboratory, in the Chemistry Department of the Federal University of Lavras, according to the methodology of the (MAPA) Ministry of Agriculture, Livestock and Supply.

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2.2 Analysis of ethyl carbamate (EC)

The method proposed by Anjos et al. (2011), Machado et al., (2013) and Santiago et al. (2014) was employed for the analysis of EC in the samples. External standardization and prior derivatization of the samples for analysis by HPLC were employed. The reagents used for analysis were the ethyl carbamate standard (Sigma aldrich), ethanol, propanol, hexane, hydrochloric acid, ethyl acetate, sodium acetate, HPLC grade acetonitrile, type I ultrapure water and 9-xanthrol (Sigma aldrich). A Shimadzu model high performance liquid chromatograph equipped with two model SPD-M20A high-pressure pumps, a model DGU-20A3 degasser, a model SIL-10AF automatic injector with an auto-sampler, a model CBM-

20A interface and a model RF-10AXL fluorescence detector (FLD). Separations were performed using an Agilent-Zorbax Eclipse AAA column (4.6 x 150 mm, 5 μm) connected to an Agilent-Zorbax Eclipse AAA pre-column (4.6 x 12.5 mm, 5 μm).

For the quantitative analysis, a 10 mg L^{-1} stock solution of the ethyl carbamate derivative in ethyl acetate was prepared. For the construction of the analytical curve, dilutions of the stock solution in 50% ethanol were prepared, and the working solutions were diluted to concentrations ranging from 5.0 to 300.0 μg L^{-1}.

Sample was transferred to an amber bottle, to which was added 4.0 mL of spirits, followed by 0.8 mL of xanthodrol solution (0.02 mol L^{-1}). After stirring, 0.4 mL of HCl (1.5 mol L^{-1}) was added with stirring, and the mixture was stirred for an additional minute. The mixture was allowed to stand for 60 minutes, filtered through a 0.45 μm polyethylene membrane (Millipore) and injected into the chromatograph.

The quantification of EC was performed using the external standard method. The excitation and emission wavelengths employed were 233 and 600 nm, respectively. The flow rate was 0.75 mL min^{-1}, and the volume of the samples and the standard injected was 20 μL. The elution was performed in a gradient type system: 0 to 5 min (40-60% B); 5 to 10 min (60-70% B); 10 to 18 min (70-80% B); 18 to 19.5 min (80-90% B); 19.5 to 25 min (90-40% B); 25 to 30 min (40% B). The mobile phase was composed of 20 mmol L^{-1} sodium acetate solution (Solvent A) and acetonitrile (Solvent B).

2.3 Analysis of Furfural and 5-Hydroxymethylfurfural

The analyses of FU and 5-HMF in spirits obtained by simple distillation from an alembic and by distillation through a fractionation column were performed according to the method described by Souza et al. (2009), with minor modifications. The standards for these compounds were purchased from Sigma-Aldrich. The mobile phase was composed of HPLC analytical grade methanol (Merck) and glacial acetic acid (J.T.Baker) and type I water obtained from a Milli-Q system.

For each of the FU and 5-HMF standards, stock solutions with a concentration of 1000 mg L^{-1} of the standards in 50% ethyl alcohol were prepared. The external standard method was used for the quantification of the compounds. The analytical curves were constructed by diluting the stock solution to furnish solutions with concentration ranges of 0.1 to 25 mg L^{-1}. The equations of the analytical curves were calculated by the method of least squares, using the detector response (area) as a function of the concentration after triplicate injections of the solutions containing the standards.

Samples and standards were filtered through a 0.45 μm polyethylene membrane (Millipore) and injected directly into the chromatographic system. Injections of the samples and standards were performed in triplicate; the identity of the analytes was confirmed by the retention time and the profiles of the peaks of the sample compared to those of the standards.

Analyses of FU and 5-HMF were performed on the same chromatograph used in the analysis of ethyl carbamate. The column used for the separations was an Agilent-Zorbax Eclipse XDB-C18 (4.6 x 250 mm, 5 μm) column connected to an Agilent-Zorbax Eclipse XDB-C18 pre-column (4.6 x 12.5 mm, 5 μm). The chromatograph was equipped with a diode array detector (DAD). The solvents used as the mobile phase were: 2% acetic acid solution in water (Solvent A) and methanol: water:acetic acid (70:28:2% v/v/v; Solvent B). Samples and standards were eluted by a gradient from 0 to 25 min (00-40% B); 25-40 min (40-55% B); 40-50 min (55-100% B); 50-60 min (100-00% B). The wavelength used was 280 nm; the flow rate was 0.8 mL min^{-1}; and the injected volume was 20 μL.

Because the procedure was performed with small modifications to the method proposed by Souza et al. (2009), the analytical parameters linearity, limit of detection, limit of quantification, precision and accuracy were determined according to the method described by Anjos et al (2011), Santiago et al. (2014) and Ribani et al. (2004) to ensure the quality of results. The linearity was determined by external standardization and formulated as a linear regression equation that was used to calculate the analyte concentration to be determined in the sample. The mathematical relationship between the signal and the concentration of the species of interest was expressed by the line equation (analytical curve) and the respective coefficients of determination (R^2). A correlation coefficient greater than 0.9900 was considered to be adequate for an ideal fit of the data to the regression line (Ribani et al., 2004; Harris, 2008).

Limits of detection (LD) and quantification (LQ) were estimated using the parameters from the analytical curve. For the determination of LD and LQ, the parameters related to each analytical curve constructed using the following mathematical relationship were considered: LD = 3 x (s/S) and LD = 10 x (s/S), where s is the estimated standard deviation from the regression line equation and S is the angular coeficiente of the analytical curve (Ribani et al., 2004; Harris, 2008).

Precision was determined from the coefficients of variation (CV) of a series of measurements using the following mathematical equation: CV (%) = (s/CMD) x 100, where s = the estimated standard deviation; CMD = mean concentration determined according to Snyder et al. (1997). The precision in the validation of methods is considered in three

different levels: repetitiveness, intermediate precision and reproducibility. In this study, the intermediate precision was used, where the analysis was performed on five different days using standard solutions for three concentration levels (0.1, 1 and 25 mg L^{-1}). The CV was estimated after successive repetitions.

Accuracy was assessed by means of recovery assays utilizing three randomly selected samples, which were fortified with three different concentrations of analyte standards at (0.5, 5 and 20 mg L^{-1}). The recovery was determined from the results obtained for each analyte using the following mathematical equation: %Recovery = [(measured concentration)/(expected concentration)] x 100.

2.4 Analysis of 2,3-butanedione

Analysis of 2,3-butanedione was accomplished using the modified method of Reche et al. (2007). Initially a derivative of the compound present in the sample was prepared and quantitatively analyzed by HPLC. The reagents used were of analytical grade, of 99.9% purity: the 2,3-butanedione standard (Sigma aldrich), methanol and acetonitrile (Merck), and 2,4-dinitrophenylhydrazine (Vetec) (Reche et al., 2007).

2,4-Dinitrophenylhydrazine (2,4-DNPH) was recrystallized three times using 2.0 g in 60 mL of boiling methanol under constant stirring until complete solubilization. The solution was filtered hot, and the filtrate was again heated to boiling. The procedure was repeated once more, and the solution was allowed to stand at room temperature until crystallization was complete.

The 2,4-DNPH derivative of 2,3-butanedione was obtained according to the method of Shriner et al. (1983). A solution of 0.4 g of 2,4-DNPH (purified) in 2 mL of sulfuric acid was prepared by adding 2 mL of water with stirring until complete solubilization. Then, 10 mL of 95% ethanol was added. In parallel, the solution of 2,3-butanedione (0.1 g) in 15 ml of ethanol was prepared. The freshly prepared 2,4-DNPH solution was added, and the resulting mixture was allowed to stand at room temperature for 10 minutes. The 2,4-DNPH derivative of 2,3-butanedione was isolated by filtration and purified by recrystallization from absolute ethanol twice. The purity was confirmed by determination of the melting point and by HPLC analysis.

For quantitative analysis, a standard solution of the 2,4-DNPH derivative of 2,3-butanedione was prepared by diluting the stock solution (1000 mg.L^{-1} in DMSO) in ethanol-

water (45:55 v/v). Six points were used to obtain the analytical curve at concentrations of 0 to 140 $mg.L^{-1}$

The samples were prepared in a 100-mL volumetric flask by dissolving 0.4 g of the purified 2,4-dinitrophenylhydrazone in methanol. In a separate flask, 1.0 mL of the 2,4-dinitrophenylhydrazone solution, 4.0 mL of the sample and 50 µL of 1.0 M perchloric acid ($HCLO_4$) were added in this order. The resulting solution was stirred at room temperature for about 45 minutes. The samples were filtered through 0.45 µm polyethylene membrane filters (Milipore), and 20 µL of the solution was injected into the HPLC for analysis (RECHE et al., 2007).

The quantitative analysis was performed by liquid chromatography using the same chromatograph as that cited in the analyzes of the other contaminants. Separations were performed using a Shim-pack VP-ODS C18 column, 25 cm x 4.6 mm (internal diameter) x 5 µm, and a Shim-pack GVP-ODS C18 pre-column, 10 mm x 4.6 mm (internal diameter) x 5 µm. The elution was performed in a gradient type system: 0-9 min. (0-60% B), 9-15 min. (60-70% B), 15-20 min (70% B), 20-30 min. (70-90%), 30-40 min (90-60% B), and 40-45 min (60% B). The mobile phase was composed of methanol: acetonitrile (80: 20% v / v) (Solvent B) and MiliQ water (Solvent A). The flow rate was 1.00 mL min-1, the injected volume of the samples and standard was 20 µL, and detection was performed using UV-vis detectionat365nm.

The quantitative conversion of ketones in the distilled alcoholic beverages to the 2,4-DNPH derivatives was ensured by the use of an excess of 2,4-dinitrophenylhydrazine. The 2,4-DNPH of 2,3-butanedione in the samples was quantified using the external standardization method (0-140 mg L-1). The analytical curve was obtained by linear regression (y = 234819x - 9086.7), plotting the peak area versus concentration, and the linear correlation coefficient was 0.9998 (Reche et al., 2007). This methodology was effective in identifying the compound 2,3 butanedione for both copper and column still drinks with external standardization using a 2,4-dinitrophenylhydrazine compound.

2.5 Statistical analysis

The experimental design was completely randomized (DIC) with 44 treatments and two replications. The results were submitted to analysis of variance using the SISVAR 9 statistical program (Ferreira, 2011). The means were compared by the Scott Knott test at the 5% level of significance ($p < 0.05$).

3. Results and Discussion

The concentrations of ethyl carbamate, 2,3-butanedione, furfural, 5-HMF and the sum of the furfural and 5-HMF found in the analyzed cachaça samples are described in Table 1.

Table 1. Chromatographic analysis of the contaminants in the distilled sugar cane spirits: ethyl carbamate, 2,3-butanedione, furfural and 5-hydroxymethyfurfural.

Samples	Ethyl carbamates (µg L^{-1})	2,3-Butanedione (µg L^{-1})	Furfural (mg/100 mL a.a.)	5-HMF mg/100 mL a.a.	FU+HMF
A1	133.73 a_2	0.66 a_1	2.39 a_1	3.24 a_2	5.63 a_2
A2	120.77 a_3	0.24 a_2	0.98 a_2	6.02 a_1	7.00 a_1
A3	131.33 a_2	0.21 a_2	0.85 a_2	2.76 a_2	3.61 a_4
A4	150.34 a_2	0.12 a_2	0.39 a_3	2.33 a_2	2.72 a_5
A5	189.48 a_1	0.18 a_2	0.92 a2	1.32 a_3	2.24 a_5
A6	145.41 a_2	0.53 a_1	<LQ	7.23 a_1	7.23 a_1
A7	143.93 a_2	0.16 a_2	2.35 a_1	2.34 a_2	4.70 a_3
A8	245.31 a_2	0.60 a_1	4.57 a_1	0.93 a_4	5.68 a_2
A9	141.52 a_2	0.31 a_2	0.52 a	0.70 a_5	1.22 a_6
A10	<LQ	<LQ	0.32 a_3	1.22 a_3	1.54 a_6
A11	235.53 a_1	0.14 a_2	0.25 a_4	2.12 a_2	2.37 a_5
A12	189.78 a_1	0.31 a_2	<LQ	0.58 a_6	0.58
A13	89.13 a_4	0.14 a_2	1.46 a_1	1.08a_3	2.54 a_5
A14	131.51 a_2	0.41 a_1	0.93 a_2	0.94 a_4	1.83 a_6
A15	144.20 a_2	0.16 a_2	<LQ	<LQ	<LQ
A16	76.21 a_4	<LQ	<LQ	<LQ	<LQ
A17	31.22 a_6	0.19 a_2	0.42 a_8	<LQ	0.42
A18	46.19 a_5	<LQ	0.02 a_9	0.03 a_{10}	0.05
A19	22.78 a_7	0.15 a_2	0.64 a_2	<LQ	0.64 a_8
A20	32.83 a_6	<LQ	0.44 a_3	0.04 a_9	0.48 a_9
A21	27.79 a_7	<LQ	0.67 a_2	0.53 a_6	1.20 a_6
A22	22.72 a_7	<LQ	<LQ	<LQ	<LQ
A23	<LQ	<LQ	0.11 a_5	<LQ	0.11 a_{13}
A24	45.14 a_5	<LQ	0.02 a_9	<LQ	0.02 a_{18}
A25	22.13 a_7	<LQ	0.07 a_6	<LQ	0.07 a_{15}
A26	14.01 a_2	<LQ	0.07 a_6	0.08 a_7	0.15 a_{13}
A27	12.67 a_2	<LQ	0.24 a_4	<LQ	0.24 a_{12}
A28	18.78 a_8	<LQ	1.24 a_1	0.07 a_8	1.28 a_6
A29	31.79 a_6	<LQ	<LQ	<LQ	<LQ
A30	<LQ	0.24 a_2	<LQ	<LQ	<LQ
A31	46.29 a_5	<LQ	0.04 a_8	<LQ	0.04 a_{17}
A32	32.06 a_6	<LQ	0.03 a_9	0.32	0.35 a_{10}
A33	<LQ	<LQ	0.83 a_2	<LQ	0.83 a_7
A34	12.34 a_2	<LQ	0.02 a_9	<LQ	0.02 a_{18}
A35	34.27 a_6	<LQ	0.04 a_8	<LQ	0.04 a_{17}
A36	<LQ	<LQ	0.05 a_7	0.63 a_5	0.68 a_8
A37	23.01 a_4	<LQ	1.24 a_1	<LQ	1.24 a_6
A38	<LQ	<LQ	0.06 a_7	<LQ	0.06 a_{16}
A39	43.32 a_5	<LQ	0.01 a_{10}	<LQ	0.01 a_{18}
A40	56.44 a_6	<LQ	<LQ	<LQ	<LQ
A41	<LQ	<LQ	<LQ	<LQ	<LQ
A42	34.04 a_6	<LQ	0.02 a_9	<LQ	0.02 a_{18}
A43	23.34 a_7	0.12 a_2	0.08 a_7	<LQ	0.08 a_{14}
A44	17.10 a_8	<LQ	<LQ	<LQ	<LQ
MAPA	210	-	-	-	5

Means followed by the same lower case letter do not differ by the Scott Knott test at the level of 5% of probability. ND = not detected, <LQ = less than the limit of quantification.

According to the data in table 1, we can see that the drinks produced in column presented high values of ethyl carbamate and that the drinks A8 and A11 are above the limit required by the legislation. The cachaças produced in a copper still, all showed values allowed by the legislation regarding the content of ethyl carbamate, and no sample exceeded its limit.

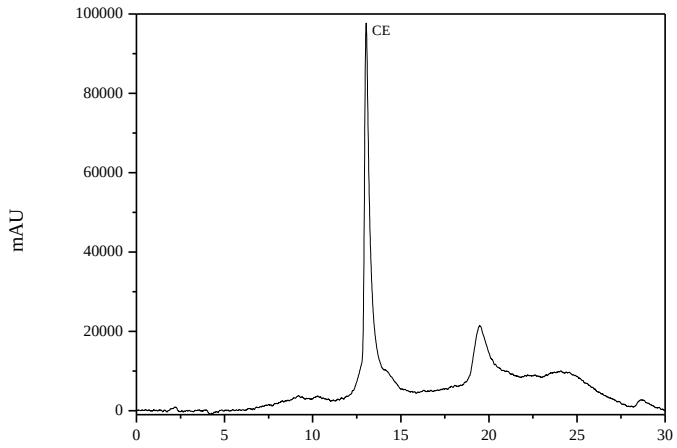
In relation to the compound 2,3 butanedione, the cachaças produced in columns were detected the presence of this compound in low concentrations, only a sample that did not present. Regarding alembic cacahas, samples A17, A19 and A30 were found to have 2.3 butanedione in low concentrations when compared to column cachaças, with the others showing no values of the compound.

The samples A1, A2 and A9 being samples produced in columns exceeded the limit of furfural and 5-hydroxymethylfurfural and the cachaças produced in copper stills are within the relevant limits according to the legislation and some did not detect the presence of these two compounds.

3.1 Analysis of Ethyl Carbamate

The HPLC chromatogram obtained for the ethyl carbamate standard is shown in Figure 1.

Figure1. Chromatogram of the ethyl carbamate (EC) standard. Concentration of the standard: 160 µg L^{-1}



Source: Autor (2020).

The linear regression of the analytical curve obtained for measurement of EC in the samples was y = 11914,70x - 47197,63 (where y = peak area and x = EC concentration). The area of the peak is correlated with the concentration of the respective standard solution, the linear correlation coefficient (R^2) being 0.99996. The limit of detection (LD) and the limit of quantification (LQ) of the method were estimated using the parameters of the analytical

curve. Their values were 1.86 and 6.23 μg.L^{-1}, respectively. These values are lower than those found by Anjos et al. (2011) (LD = 3.93 μg L^{-1} and LQ = 13.09 μg L^{-1}); Machado et al. (2013) (LD = 6.39 μg L^{-1} and LQ = 21.32 μg L^{-1}); Mendonça (2016) (LD = 2.48 μg L^{-1} and LQ = 8.26 μg L^{-1}); and Santiago et al. (2014) (LD = 3.24 μg L^{-1} and LQ = 10.83 μg L^{-1}).

The concentrations of ethyl carbamate in the samples obtained from alembic stills and fractionation columns were different. The concentrations of ethyl carbamate in column spirits ranged from <LQ to 245.31 μg L^{-1}, whereas those from alembics varied from <LQ to 76.21 μg L^{-1}. Only two spirits, L8 and L11 contained concentrations of 245.31 μg L^{-1} and 235.53 μg L^{-1}, respectively, differing significantly from the other samples and exceeding the legal limit, which is 210 μg L^{-1}.

The ethyl carbamate concentrations found for all the alembic samples were within the legal limit. The lowest concentrations of EC were observed in the following samples: A23, A30, A33, A36 and A41, where the concentrations were below the LQ of the method. All the alembic samples contained concentrations below the limit established by the Brazilian legislation, and these results are similar to those found by Barcelos et al. (2007), Masson et al. (2014), Machado et al. (2013) and Anjos et al. (2011), who did not find levels of ethyl carbamate above the legal limit in spirits from the south of the state of Minas Gerais.

Barcelos et al. (2007) evaluated cachaça samples from three different regions of the state of Minas Gerais (southern Minas Gerais, Zona da Mata and JequitinhonhaValley), and the concentrations of EC ranged from undetected to 700 μg L^{-1}. Among the regions studied, only samples from the Jequitinhonha Valley contained concentrtions of EC higher than those established by the ABPM. Masson et al. (2007) studied cachaças produced in small and medium-sized stills from the northern and southern regions of Minas Gerais and found values ranging from 22 to 980 μg L^{-1}. The authors reported that the EC concentrations found in the cachaça do not correlate with the alcoholic strength, acidity or copper concentrations in the samples. Although some authors believe that the distillation of cachaça in copper stills promotes the metal-catalyzed formation of EC (Aresta, Boscolo, Franco, 2001; Andrade-Sobrinho et al., 2002; Bruno et al., 2007; Masson et al., 2014), other authors demonstrated that no correlation of copper with EC in cachaça exists (Barcelos et al., 2007; Machado et al., 2013; Zacaroni et al., 2011; Anjos et al., 2011, Santiago et al., 2014; Mendonça et al., 2016).

Anjos et al. (2011) confirmed that storage of the beverage in both oak barrels and glass containers influenced the formation of ethyl carbamate, leading to a significant increase in the concentration of this contaminant. For the authors, the presence of light also has an influence,

because 13.63 μg L^{-1} of EC was detected in the cachaça stored in glass containers as early as the second month of storage, whereas the same concentration was only observed in the cachaça stored in the oak barrels in the ninth month. These data corroborate those obtained in this study. The aged alembic cachaças contained low concentrations of EC. Santiago et al. (2014) periodically followed the chromatographic profile of EC in the production process and the aging of cachaça in amburana barrels. The authors observed that the concentration of EC throughout the production process and in the aging stage was lower than the legal limit for this compound.

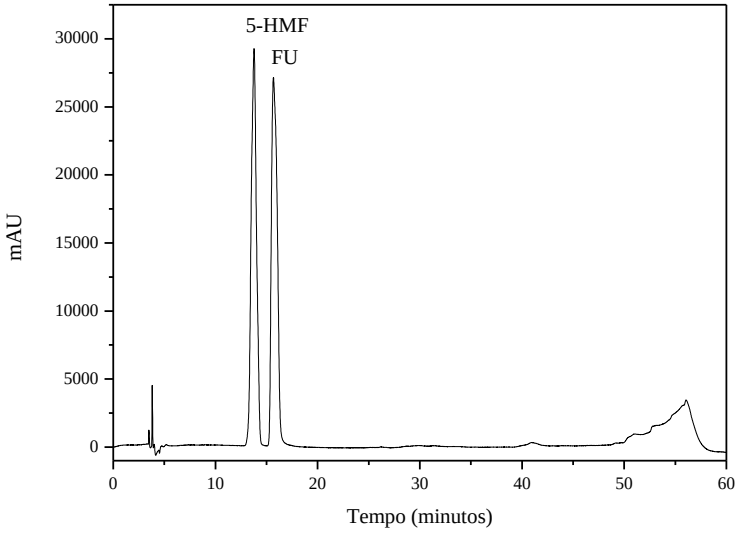
Baffa Júnior (2011) demonstrated the existence of correlations between the concentration of this contaminant and the parameters involved in the production process and, mainly, during the distillation process, such as the material of which the still is composed (copper or stainless steel), type of heating (direct or indirect), fraction cuts (head, heart and tail), and reflux rate during the process. Serafim et al. (2012) analyzed samples of cachaças distilled through stainless steel columns and observed that these beverages generally have concentrations of ethyl carbamate higher than that of cachaça obtained from copper alembics with the same wort. The sums of the carbamate concentrations of the "heart" and "tail" fractions of distillates from alembics are generally compatible with the contents of the column distillate. Thus, the cachaças obtained from copper stills tend to have lower concentrations of carbamate than the cachaça distilled through columns because of the fraction cuts. The authors state that the presence of ethyl carbamate occurs mainly in the following order of the distillates — "head"> "heart"> "tail". Therefore, it is foreseeable that the "head" fraction should have a higher concentration of ethyl carbamate because it has a higher concentration of alcohol. The results found by Serafim and collaborators (2012) corroborate the results obtained in this study, in which higher concentrations of EC were found in the column cachaças than in the alembic cachaças.

Galinaro and Franco (2011) demonstrated that there is a gradual increase in the concentration of ethyl carbamate in spirits from the same wort and distilled in alembic and column stills during the first week after distillation. After this period, stabilization occurs in the content of this compound. Thus, the ethyl carbamate formed after distillation is due to the presence of potential precursors in the various fractions of the distillate. However, the authors state that the differences between the ethyl carbamate concentrations observed in the column distillates and in the alembic fractions are due to the distillation process and independent of the fermentation process.

3.2 Analysis of furfural and 5-hydroxymethylfurfural

The chromatographic profile of the standard solution of FU and 5-HMF showing the separation of the two compounds is presented in Figure 2

Figure 2. Chromatogram of the furfural (FU) and 5-hYdroxymethylfurfural (5-HMF) (1mg L^{-1}) standard.



Source: Autor (2020).

The retention time for furfural was 15.567 ± 0.12 minutes and that for the 5-HMF of 13, 658 ± 0.14 minutes, which agree with the values found by Souza et al. (2009). Under the chromatographic conditions employed, no interfering substances were observed at the retention times of FU and 5-HMF. The linearity of the method was evaluated by the estimation of the coefficients of determination referring to the equations of the curves, obtained by linear regression. For furfural, a linear regression coefficient of 0.9999 was obtained, and a linear regression coefficient of 0.99999 was obtained for 5-HMF.

The precision was estimated from the repeatability (coefficients of variation (CV). The CVs were obtained from the results obtained by five repetitions, which varied from 1.01% at the concentration of 0.1 mg L^{-1}, 1.98% at the concentration of 1.0 mg L^{-1}, and 3.88% at the concentration of 25 mg L^{-1} for FU. The results obtained from the CV for 5-HMF ranged from 1.23% at the concentration of 0.1 mg L^{-1}, 1.14% at the concentration of 1.0 mg L^{-1} and 3.68% at the concentration of 25 mg L^{-1}. In general, the values found for the CV are below the 5% limit for the two compounds analyzed in the repeatability tests, as is recommended in the

literature (RIBANI et al., 2004; HARRIS, 2008). Therefore, one can affirm that the method presents adequate precision for the two compounds analyzed in cachaças.

The detection limits for FU and 5-HMF were 0.087 mg L^{-1} (0.017 mg/100 mL a.a.) and 0.058 mg L^{-1} (0.011 mg/100 mL a.a.), respectively. For the quantification limits, 0.91 mg L^{1} (0.058 mg/100 mL a.a.) was found for furfural, and 0.193 mg L^{-1} (0.039 mg/100 mL a.a.) was found for 5-HMF.

The accuracy of the analytical method was assessed by means of recovery assays in which the concentrations of FU and 5-HMF were calculated from increasing peak areas after the addition of a known quantity of the standards to three randomly selected samples: A12, A13 and A27. The concentrations used for furfural and 5-HMF were 0.5; 5.0 and 20.0 mg L^{-1}. A 0.5-mg L^{-1} aliquot of furfural was added to three samples, and recoveries of 98%, 83% and 108% recovery was obtained. Recoveries of 96%, 94% and 78% were obtained when a 5.0 mg L^{-1} concentration was used, and of 97%, 99% and 98%, respectively, with a concentration of 20 mg L^{-1}. The same procedure was repeated for 5-HMF and yielded the following results: concentration of 0.5 mg L^{-1} (recovery of 95% 83% and 74%); concentration of 5.0 mg L^{-1} (recovery of 93%, 97% and 81%) and for the concentration of 20.0 mg L^{-1} (recovery of 107%, 100% and 98%, respectively). The acceptable limits for recovery are between 70 and 120%. Thus, a good recovery was obtained for the two compounds analyzed, whose average values were within the acceptable limits (Ribani et al., 2004; Harris, 2008). Recovery percentages similar to those found in this study were obtained by Anjos et al. (2011) using the same method for the quantification of phenolic compounds.

None of the FU or 5-HMF concentrations in the alembic cachaça samples exceeded the legal limits (Table 1). However, three samples obtained by column distillation -— A1, A2 and A8 — exceeded the 5.0 mg/100 mL a.a. limit (5.63, 7.00 and 5.68 mg/100 mL a.a., respectively).

Furfural and 5-HMF are organic contaminants whose presence is undesirable in the beverage. The high temperature, associated with the low pH of the wort, leads to hydrolysis of polysaccharides of the bagasse (cellulose, hemicellulose, pectin and others) and dehydration of the sugars to form 2-furfural and 5-hydroxymethyl-2-furfural (5-HMF) Pentoses form furfural as the principal degradation product, whereas hexoses form 5-HMF. Other factors, such as the aging of the beverage under irregular conditions, the addition of caramel and pyrogenation of the organic matter deposited on the bottom of the stills might also contribute to the increase in the concentrations of these components (FARIA et al., 2003; ZACARONI et al. 2011).

Masson et al. (2007) studied the formation of furfural and 5-HMF using raw and burned cane for the production of cachaça and showed that the burning and the reheating of sugar cane before grinding influenced the appearance of these compounds. In burning the sugar cane straw, the sugar exudate becomes an excellent adhesive for the combustion residue, solid particles of soil, minerals and others. In processing the cane, these residues are transferred to the broth and, in suspension, are transferred to the dornas and later to the alembic, where the organic matter is transformed into furfural and 5-HMF.

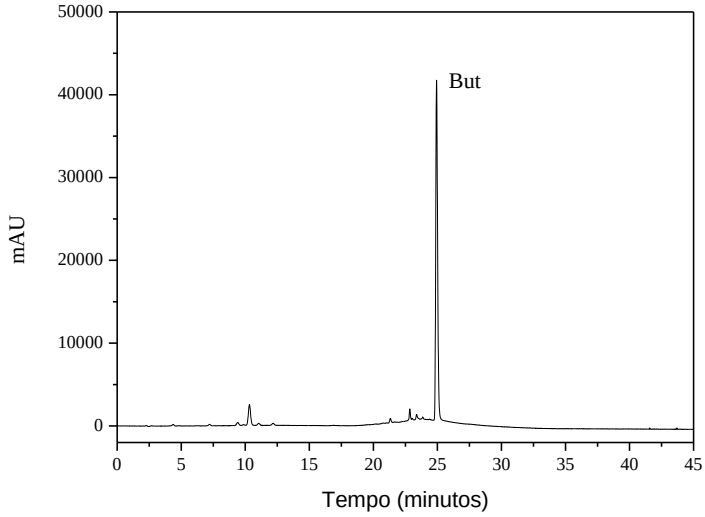
According to Serafim et al. (2012), the concentrations of aldehydes were highest in the "head" fraction, with the exception of 5-HMF and FU, whose concentrations were higher in the "heart" and "tail" fractions of the distillate. This fact might be related and their respective boiling temperatures. Reche et al. (2007) analyzed 27 samples of cachaça and observed that the samples distilled in a column had a higher concentration of acetic acid and 5-HMF than the alembic cachaças.

Pinheiro et al. (2010) randomly analyzed 16 samples of alembic and column cachaças. The concentrations of FU and 5-HMF were within the legal limits. The values were lower than 1.0 mg/100 mL a.a. None of the samples was produced from burned sugar cane, no residual sugars or polysaccharides from bagasse were present in sugarcane, and no caramel was added.

3.3 Chromatographic analysis of 2,3-Butanedione

The chromatogram obtained for the 2,3-butanedione standard is presented in Figure 2.

Figura 3. Chromatogram of the 2,3 butanedione 2,4- dinitrophenylhydrazone standard. Concentration of the standard: 140 µg.L^{-1}



Source: Autor (2020).

The mean retention time for the compound was 25 minutes. The concentration of 2,3-butanedione was determined by the construction of the analytical curve, obtained by linear regression (y = 234819x - 9086.7) of a plot of peak área versus concentration. The linear correlation coefficient was 0.99998. The values for the limit of detection (LD) and quantification (LQ) were 0.054 and 0.0182 μg L^{-1}, respectively.

A predominance of 2,3-butanedione in the spirits can be observed in Table 3, and the samples with the highest concentrations of this compound were samples L1 and L6, with 0.66 μg L^{-1} and 0.53 μg L^{-1}, respectively. The concentrations were low in alembic liquor samples, and many samples contained concentrations below the limit of quantification.

Moreira, Neto & Maria (2012) reported that ketones can be present in several fermentation processes because of variations in temperature, nutrients present in the must, the amount of oxygen available and the type of microorganism. These compounds can be present in fermented and distilled alcoholic beverages and often contribute to the taste and aroma of these beverages, including Brazilian sugar cane spirits. However, prolonged inhalation of these compounds can cause irritation of the mucous membranes, headaches, seizures, narcotic effects and can result in coma. These authors have developed some studies on sugar cane spirits produced in copper and stainless steel stills using HPLC and by derivatization of ketones to obtain 2,4-dinitrophenylhydrazones. The 2,3-butanedione (mean value of 4.3 mg L^{-1}) was the ketone presente in the highest concentration. The authors also observed that the average content of this product was not statistically different in cane spirits

produced in copper alembics and from that distilled through a stainless steel column, a fact that corroborates the results found in this study.

According to Nakashimada, Kanai and Nshio (1998), batch fermentations fed with excess nutrients based on carbohydrates can influence the concentration of acetoin during the fermentation process. Another possible factor that might influence the formation of 2,3-butanedione during fermentation is the supply of oxygen. The authors performed an experiment under the same fermentation conditions (initial sugar concentration, pH, temperature), but the stirring speed was varied from 500 to 750 rpm after the activation of the yeast to initiate the fermentation process. They observed an increase in concentration of 2,3-butanedione at the end of the fermentation process.

Perego et al. (2003) stated that ketone production is associated with the aeration rate. The higher the aeration rate, the greater the amount of ketone produced. The effect of temperature also interferes with ketone production. The authors found that there was an increase in the final concentration of the product at temperatures above 37 °C. The authors further reported that the production of ketones during yeast fermentation is dependent on temperature. When the temperature increases, the rate of decomposition of acetolactate also increases, and more 2,3-butanedione is produced. The authors state that various changes in temperature may occur in the production of fermented and distilled beverages that lead to the production of ketones.

4. Conclusion

There were differences with regard to the chromatographic parameters (ethyl carbamate, 2,3-butanedione, furfural and 5-HMF) in all the samples studied.

The concentrations of ethyl carbamate in spirits distilled from an alambic were below the maximum limit allowed by legislation, and in some samples, it was not detected. For spirits distilled through a fractionation column, the concentrations ranged from less than the limit of quantification to 245.31 μg L^{-1}. The concentrations of 2,3-butanedione in column spirits were higher than in alambic liquors. The chromatographic method was validated and proved to be efficient for the simultaneous analysis of the furfural and 5-hydroxymethylfurfural in samples of sugar cane spirits. Column spirits had higher concentrations, ranging from 5.63 to 7.00 mg/100 mL a.a. and alembic spirits contained concentrations below the legal limits. Thus, the spirits obtained by column distillation contained higher concentrations of the contaminants than liquor obtained from an alembic, a

fact that induces the industrial producers to use greater rigor during process of manufacturing the beverage.

Based on the results of this shield in the production of cachaças in copper and column stills, it requires greater monitoring of the quality of the drink, mainly in the formation of organic contaminants that disqualifies the drinks under the legislation in force. Therefore, more rigorous concepts in production should be improved, mainly in the fermentation and distillation processes and elucidate the mechanism of formation of ethyl carbamate, which is considered a highly toxic compound, causing serious problems in the consumer's health.

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